

# LONG-TERM BIOMONITORING OF SOIL CONTAMINATION USING POPLAR TREES: ACCUMULATION OF TRACE ELEMENTS IN LEAVES AND FRUITS

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*Phytostabilization aims to immobilize soil contaminants using higher plants. The accumulation of trace elements in *Populus alba* leaves was monitored for 12 years after a mine spill. Concentrations of As and Pb significantly decreased, while concentrations of Cd and Zn did not significantly over time. Soil concentrations extracted by  $\text{CaCl}_2$  were measured by ICP-OES and results of As and Pb were below the detection limit. Long-term biomonitoring of soil contamination using poplar leaves was proven to be better suited for the study of trace elements. Plants suitable for phytostabilization must also be able to survive and reproduce in contaminated soils. Concentrations of trace elements were also measured in *P. alba* fruiting catkins to determine the effect on its reproduction potential. Cadmium and Zn were found to accumulate in fruiting catkins, with the transfer coefficient for Cd significantly greater than Zn. It is possible for trace elements to translocate to seed, which presents a concern for seed germination, establishment and colonization. We conclude that white poplar is a suitable tree for long-term monitoring of soil contaminated with Cd and Zn, and for phytostabilization in riparian habitats, although some caution should be taken with the possible effects on the food web.*

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**KEY WORDS:** Salicaceae, trace elements, bioindicators, *Populus alba*, Guadamar, phytostabilization

## INTRODUCTION

Phytostabilization is a remediation technique that uses plants to immobilize soil contaminants. The establishment of a vegetation cover is undoubtedly the most useful and widespread method of physically stabilizing mine wastes and reducing metal pollution effects (Bargagli 1998). A vegetative cover plays a vital role in stabilization by reducing the water flux through soil profile and mechanically stabilizing the soil through root growth, while also reducing the movement of soil and the associated contaminants. Poplar trees, as well as deep-rooted plants with high transpiration rates, can effectively decrease the migration of contaminants from surface water into groundwater (Bolan *et al.* 2011). In addition, poplars (and other trees belonging to the *Salicaceae* family) are well-known

by their ability to accumulate Cd and Zn in their leaves, making them suitable for the phytoextraction of these elements from contaminated sites (Robinson *et al.* 2000), and also as biomonitors of soil contamination (Madejón *et al.* 2004, 2006).

In phytostabilization, biomarkers are plants that provide information concerning the environmental quality at a contaminated site (Market *et al.*, 2003). Long term soil contamination may be assessed by using plants as biomonitors, especially in areas where sampling and chemical analysis of soil samples may be challenging and expensive (Madejón *et al.* 2006; Bone *et al.* 2010). The use of trees as biomarkers is an inexpensive and efficient way to assess trace element bioavailability (Madejón *et al.* 2006; Bone *et al.* 2010).

Plants suitable for phytostabilization must be able to survive and reproduce in contaminated soils (Bolan *et al.* 2011). In many plants, physiological barriers impede the translocation of metals to seeds during their maturation (Ernst, Vekleij, and Schat 1992). For example, seeds from sunflower plants growing in contaminated soil had a lower concentration of Arsenic (As) and a lower concentration of lead (Pb) than the blades of the same plant (Madejón *et al.* 2003). Poplar trees have the ability to accumulate high levels of Cd and Zn in their leaves (Robinson *et al.* 2000; Madejón *et al.* 2004; Lepp and Madejón 2007) and it is unknown whether these contaminants will also be transferred to the plants' fruits and seeds.

In a previous study (Madejón *et al.* 2004), the leaves of *Populus alba* L. were used as biomonitors of the extent of Cd and Zn soil contamination after a mine spill in the Guadiamar basin. Thirteen years after the spill and the phytoremediation of the area, Cd still persists at worrying concentrations (Domínguez *et al.* 2008; Madejón, Domínguez, and Murillo 2012). In order to determine future management and remediation actions it is necessary to determine the bioavailability of Cd, and of trace elements in general, to the plants chosen for phytoremediation (Chapman 2012). Therefore this study was conducted at the same spill site as reported by Madejón *et al.* (2004).

The main objectives of this study were to evaluate the use of *Populus alba* as a biomonitor and to determine its potential to phytostabilize soil containing potential toxic trace elements (Cd, Zn, As, and Pb). In order to determine the ability of *P. alba* to phytoextract trace elements, the bioavailability of trace elements to the leaves of *P. alba* was assessed over 12 years. In addition, the concentration of trace elements in fruiting catkins of *P. alba* was determined to assess the potential effects that trace element contaminated soil has on reproduction.

## MATERIAL AND METHODS

### Site and Species Study

In 1998, the Aznalcóllar mine spill contaminated approximately 55 km<sup>2</sup> of the Guadiamar valley with trace elements (As, Cd, Cu, Pb, Tl, and Zn) (Cabrera *et al.* 1999). This area was subjected to a large-scale phytomanagement project, including clean up of sludge and topsoil, addition of soil amendments, and plantation of native shrubs and trees, and consequently protected as the "Guadiamar Green Corridor" (CMA 2003; Domínguez *et al.* 2008).

The climate of the studied zone is typically Mediterranean, with mild rainy winters (about 500 mm mean annual rainfall, Domínguez *et al.* 2008) and very hot and dry summers, with maximum values of solar radiation exceeding 1000 Wm<sup>-2</sup>. The mean annual daily temperature is approximately 17°C, with a maximum temperature of 33.5°C in July and a minimum temperature of 5.2°C in January. The predominant soils in the area belong to the great groups: Xerofluvent, Xerochrept, Haploxeralf and Rhodoxeralf (Cabrera *et al.* 2005).

White poplar (*Populus alba* L.) is a common species in the riparian forest of the Guadamar River, and it has been widely planted in the spill-affected area, which mostly consists of former agricultural lands (Madejón *et al.* 2004). In general, poplar species are suitable for phytoremediation because they are relatively tolerant to soil contamination, grow fast and have deep roots (Robinson *et al.* 2000, 2007; Bolan *et al.* 2011).

**Sampling strategy.** In a previous experiment, samples of *Populus alba* leaves were collected from five contaminated sites of the Guadamar valley in 1999 (Madejón *et al.* 2004). Two sites: Aznalcázar (AZ, 37° 18' 12", N 6° 15' 38" W) and Doblas (DO, 37° 23' 45" N, 6° 13' 35" W) were selected for additional leaf sampling. In October of 2000, 2001, 2005, and 2010, poplar leaves were taken from five trees per site. Using a spiral auger (2.5 cm diameter), soil samples were taken from the root-zone of each tree, about 2 m from the trunk, and at two depths (0–20 and 20–40 cm). At each tree, three soil subsamples were collected at each depth.

In the spring of 2011, fruiting catkins and leaves were also collected from five trees at each site (AZ and DO). For comparison, fruiting catkins and leaves were also collected from an uncontaminated site, Rivera de Huelva (RHU, 37° 29' 4.8" N, 6° 01' 34.0" W).

## Plant and Soil Analysis

Plant material was partitioned for analysis into leaves and reproductive structures (for spring 2011 samples). Additionally, catkins were separated into green capsules (fruits) and the central stem or rachis. Samples were washed twice with distilled water for approximately 10 seconds, dried at 70°C for at least 48 h, and ground by using a stainless-steel mill. Trace elements (As, Cd, Cu, Fe, Mn, Ni, Pb, and Zn) and nutrients (Ca, K, Mg, N, P y S) were determined by wet oxidation with concentrated HNO<sub>3</sub> under pressure in a microwave digester. The analysis of trace metals in the digests was performed by ICP-MS (inductively coupled plasma-mass spectroscopy). Analysis of mineral nutrients in the extracts was performed by ICP-OES (inductively coupled plasma spectrophotometry). The accuracy of the analytical method was determined using two plant reference materials: CRM 279 (sea lettuce) and NCS DS 73348 (poplar leaves) (Madejón *et al.* 2004). Recovery rates for reference plant samples were between 90 and 110%.

Soil samples were oven-dried at 40°C, for at least 48 h, and crushed to pass a 2 mm sieve, and then ground to < 60 mm for trace element analysis. Total content of trace elements was determined by ICP-OES, after digesting the samples with a mixture of concentrated HNO<sub>3</sub> and HCl ('aqua regia'). Available trace element in soils (in the soil fraction <2 mm) was determined by an extraction with 0.01 M CaCl<sub>2</sub> in a relation 1 (soil sample)/10 (0.01 M CaCl<sub>2</sub> extracts) after shaking for 3 h (Houba *et al.*, 1996) and analyzed via ICP-OES.

The transfer coefficient (TC) is defined as the ratio of metal concentration in the plant to the total metal concentration in the soil (Adriano 2001). The TC was calculated in order to determine the concentration of trace elements that was transferred from the soil to aboveground plant tissue. In this study we calculated TC for leaves and for fruits separately using soil concentrations obtained from soil sampled at depth 0–20 cm.

## Statistical Analyses

The temporal variation of the concentration of elements in poplar leaves was analyzed by ANOVA, based upon the year sampled and the site location. For the 2011 plant samples, site locations and plant fractions were used as fixed factors in the ANOVA analysis. When necessary, complementary analysis of the *T-student* test for paired means was carried out.

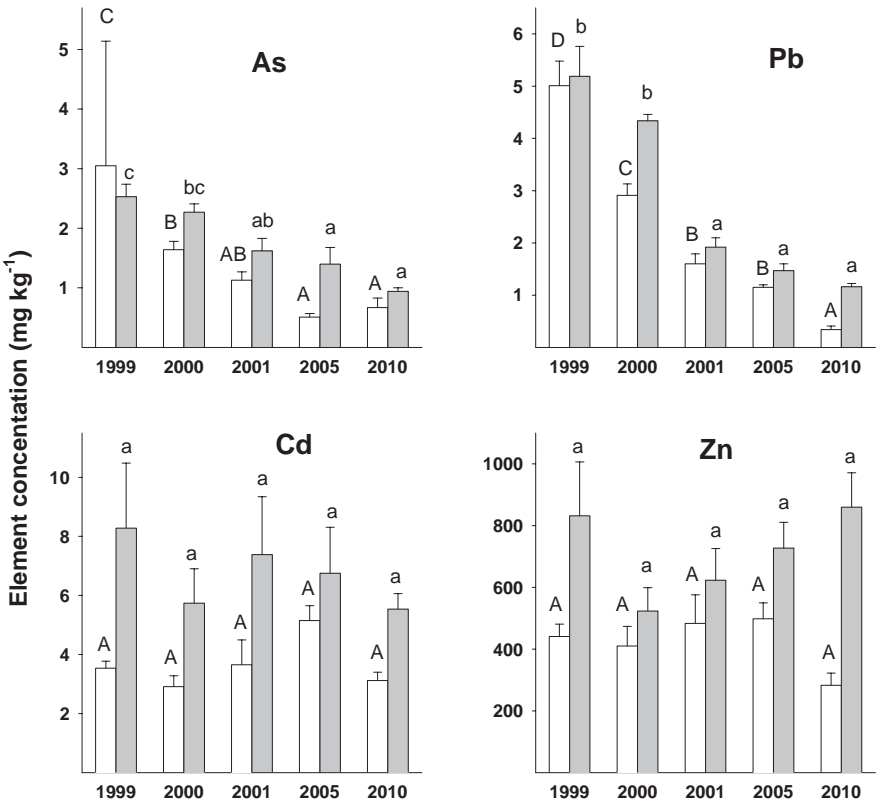
Normality of the data was tested prior to analysis. Significant differences of all variables between the different years, sites and plant fractions were established by post-hoc Tukey's test. Correlation analyses for each element between the different fractions were calculated. The significance levels reported are based on Pearson coefficients.

To investigate the global pollution trends among sites and years, a principal component analysis (PCA) was performed using the concentration of eight trace elements (As, Cd, Cu, Fe, Mn, Ni, Pb, Zn) in the leaves of 10 poplar trees over 5 sampling years (total of 50 cases). Previously, variables were transformed by square root to obtain a normal distribution. All statistical analyses were carried out with the program SPSS 15.0 for Windows.

## RESULTS

### Monitoring long-term evolution of trace elements using poplar leaves

Temporal patterns of trace elements in poplar trees are shown in Figure 1 and Table S1. The less mobile elements, As and Pb behaved differently than Cd and Zn. From 1999–2010, As progressively and significantly reduced its leaf concentration from 3.05 mg



**Figure 1** Temporal variation in trace element (As, Pb, Cd, and Zn) concentrations in leaves of *Populus alba* L. at two sites: 'Aznalcázar' (AZ, white columns) and 'Doblas' (DO, grey columns), during the period 1999–2010 (autumn samplings). For each element and site bars with the same letter do not differ significantly ( $p < 0.05$ ). Mean values  $\pm$  standard errors are expressed as  $\text{mg kg}^{-1}$  of dry matter.

**Table 1** Extractable (by CaCl<sub>2</sub>) concentrations (mean value ± standard error; mg kg<sup>-1</sup>) of trace elements in the two studied soils at depth 0–20 and 20–40 cm.

Year	Soil	Depth	As	Cd	Pb	Zn
1999	AZ	0–20	<DL	0.01 ± 0.002	<DL	3.00 ± 0.99
		20–40	<DL	0.03 ± 0.009	<DL	0.95 ± 0.41
	DO	0–20	<DL	0.24 ± 0.10	<DL	49.8 ± 20.4
		20–40	<DL	0.29 ± 0.10	<DL	55.1 ± 22.7
2010	AZ	0–20	<DL	0.01 ± 0.002	<DL	0.20 ± 0.08
		20–40	<DL	0.01 ± 0.002	<DL	0.10 ± 0.01
	DO	0–20	<DL	0.51 ± 0.22	0.25 ± 0.09	79.0 ± 35.0
		20–40	<DL	0.48 ± 0.16	0.14 ± 0.03	65.7 ± 21.1

DL: detection limit

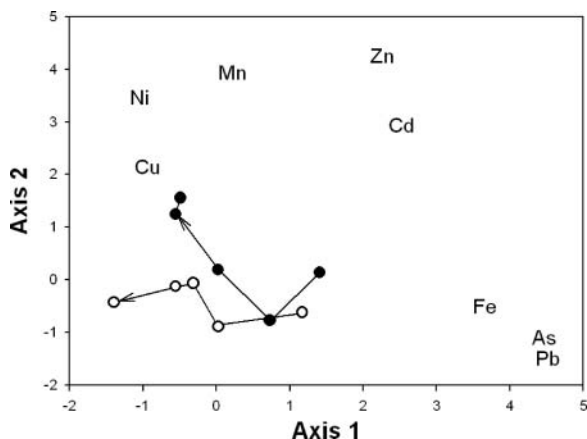
kg<sup>-1</sup> to 0.66 mg kg<sup>-1</sup> at site AZ and from 2.53 mg kg<sup>-1</sup> to 0.95 mg kg<sup>-1</sup> at site DO. Similarly Pb values decreased from 5.00 mg kg<sup>-1</sup> to 0.33 mg kg<sup>-1</sup> in AZ and from 5.19 mg kg<sup>-1</sup> to 1.15 mg kg<sup>-1</sup> at site DO. However, Cd and Zn concentrations remained comparatively high and did not differ significantly over time (Table S2). Concentrations of Cd and Zn at site AZ were significantly different from Cd and Zn concentrations at site DO.

Previously, Madejón *et al.* (2004) reported that soil at both sites (AZ and DO) in 1999 had a neutral pH, and trace element concentrations (extracted by ‘aqua regia’) of approximately 111 mg kg<sup>-1</sup> of As, 4 mg kg<sup>-1</sup> of Cd, 305 mg kg<sup>-1</sup> of Pb and 583 mg kg<sup>-1</sup> Zn.

Available contents of As and Pb in soils (0.01M CaCl<sub>2</sub> extraction) were below the detection limit, with exception of 2010 Pb samples at DO site (Table 1). The measurable concentrations of Cd and Zn did not show a definite temporal trend with respect to the year sampled and the site location. Although, soil samples analyzed for Zn at site AZ were significantly different between years.

Figure 2 shows the pollution multivariate trends in poplar leaves during the 12 years of study. The first PCA axis explained 33.7% of the variance and was associated with a global trend of five elements (Pb, As, Fe, Cd, and Zn), while the second PCA axis, with 30.7% of the variance, separated Pb, As and Fe (negative scores) from Cd and Zn (positive scores). The evolution of trace elements in poplar leaves with time was represented by the changes in the PCA-space location of mean values per site in each of the five sampling years. In general, samples of both sites moved towards PCA coordinates indicating lower concentrations of Pb and As (negative scores of axis 1), Cd and Zn. However, with respect to the second PCA axis, samples of the two sites diverged with time. Soil samples from the DO site increased in Zn and Mn (positive scores of axis 2) while those from the AZ site remained similar with time.

The transfer coefficients (TCs) between the soil and *P. alba* followed similar temporal trends as leaf concentrations. A significant ( $p < 0.05$ ) decreasing TC trend for As and Pb was observed, while the TC trend for Cd and Zn did not significantly differ with time. Transfer coefficients for Cd were significantly higher than TCs for Zn at site DO in 1999 and at site AZ in 2010. The transfer coefficients for Cd and Zn were both much higher than those of As and Pb (Figure 3). These results suggest that Cd and Zn are translocated into *P. alba* at a higher rate than As and Pb.

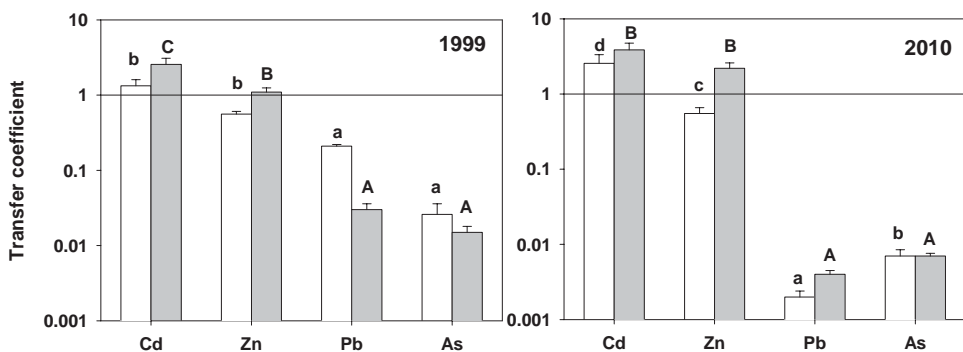


**Figure 2** Ordination of poplar leaf samples by the two first axes of principal component analysis (PCA), according to their concentration of eight trace elements. Each circle represents the mean value for several trees in a single year sampling; in black for Doblas site and in white for Aznalcázar site. Arrows indicate the temporal trend direction from 1999 to 2010 samples. Scores of the elements in the PCA space have been multiplied by five, for clarity.

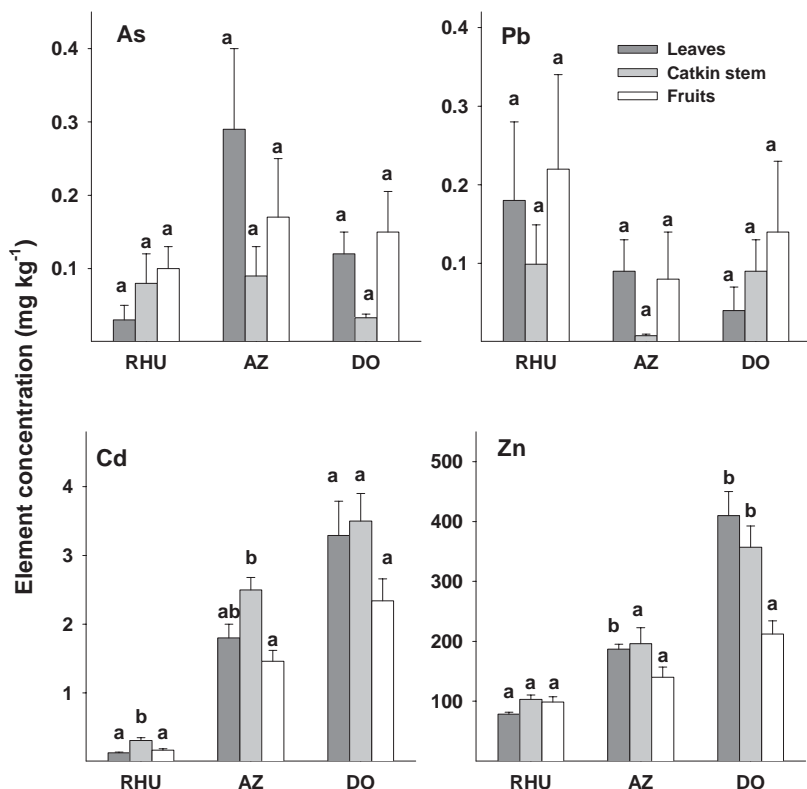
### Allocation of Trace Elements and Nutrients in Poplar Leaves and Fruits

Concentrations of As and Pb in all poplar fractions were extremely low ( $< 0.1$ – $0.3 \text{ mg kg}^{-1}$ ; Figure 4) compared to concentrations measured in autumn leaves (approximately  $1 \text{ mg kg}^{-1}$ , Figure 1), which had the largest concentration of As and Pb. In poplar fruits, the accumulation of As and Pb from contaminated soils was not significantly different from the non-contaminated site (RHU) ( $p < 0.23$ ), despite the significant differences in leaf concentration ( $p < 0.05$ , AZ). The ANOVA tests for As and Pb did not show significant differences between plant organs and sites (Table S3).

In spring 2011, a consistent accumulation of Cd and Zn was measured in the catkin stems and fruits (green capsules). Maximum concentrations of Cd and Zn in catkin stems were  $3.5 \text{ mg kg}^{-1}$  and  $360 \text{ mg kg}^{-1}$ , respectively at DO site (Figure 4). Within the catkins,



**Figure 3** Transfer coefficients (in 1999 and 2010), defined as element concentration in leaves divided by the total element concentration in soils (at 0–20 cm depth) in AZ site (white bars) and DO site (grey bars) for poplar leaves. Mean values  $\pm$  standard error, expressed in log scale. For each year and site bars with the same letter do not differ significantly ( $p < 0.05$ ).



**Figure 4** Trace element concentration in different plant fractions of *Populus alba* L. (spring sampling 2011). For each site, bars (fractions) with the same letter do not differ significantly ( $p < 0.05$ ). Mean values  $\pm$  standard errors are expressed as  $\text{mg kg}^{-1}$  of dry matter.

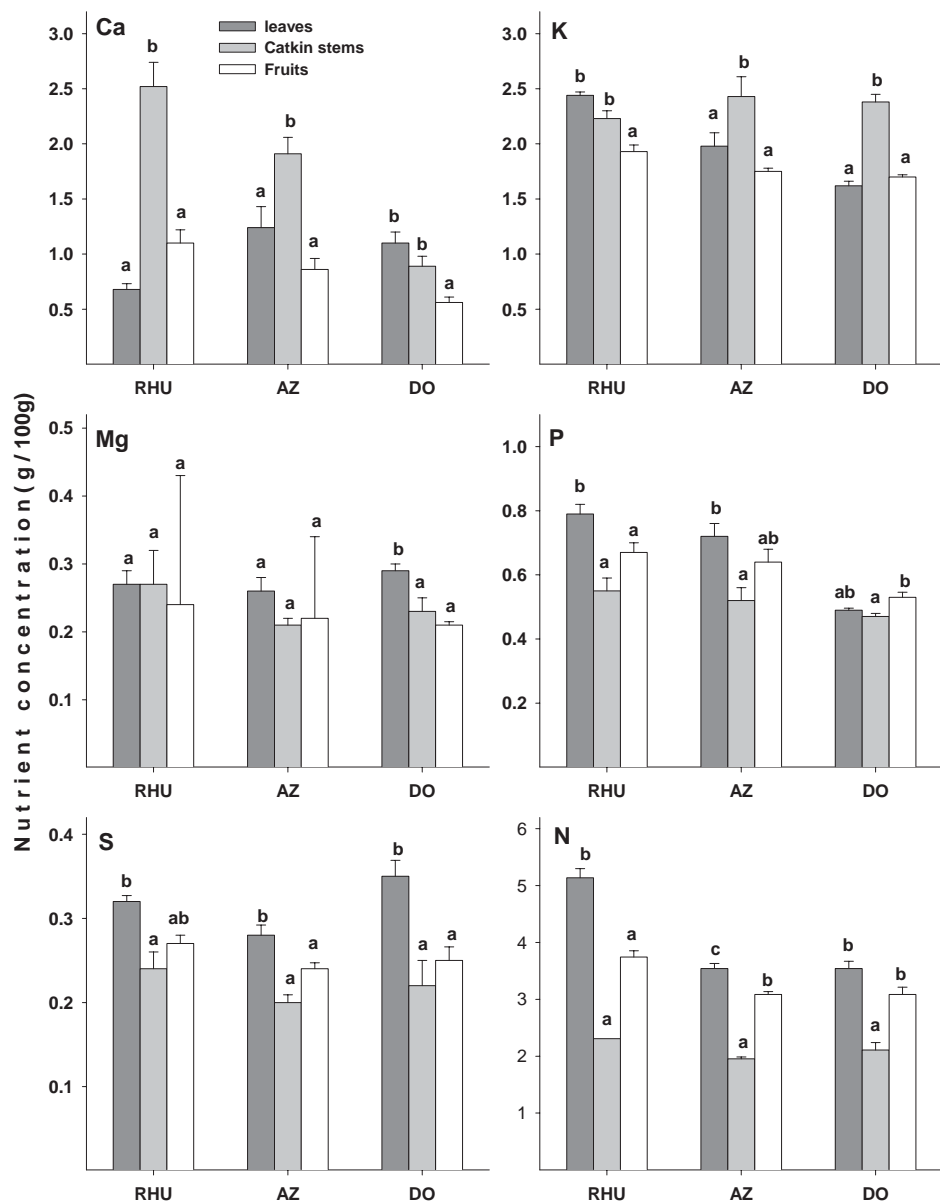
Cd concentrations were significantly lower in the fruits when compared to the stems at site AZ and Zn was significantly lower in the fruits than the stems at site DO (Figure 4). When comparing the three organs (leaves, catkin stems and fruits) significant correlations were found between concentrations of Zn and Cd ( $r = 0.96$   $p < 0.001$ ).

Nutrient concentrations from the leaves, catkin stems and fruits are shown in Figure 5. There were significant differences between sites and organs for Ca, K, P, and S (Table S3). The non-contaminated site (RHU) had significantly more nitrogen in *P. alba* fruits and leaves, significantly more K in the leaves, and significantly more Ca in the fruits.

During the spring 2011 sampling, the transfer coefficient for Cd from soil to fruits at RHU tended to be greater than from soil to leaves, with the opposite trend at the contaminated sites (Figure 6). In general, the TC values for Cd (1.5–2.5) were greater than those of Zn (0.5–1.5). In the case of Zn, the TC values from soil to both leaves and fruits were higher at the uncontaminated site (RHU) than at the contaminated sites, AZ and DO (Figure 6).

## DISCUSSION

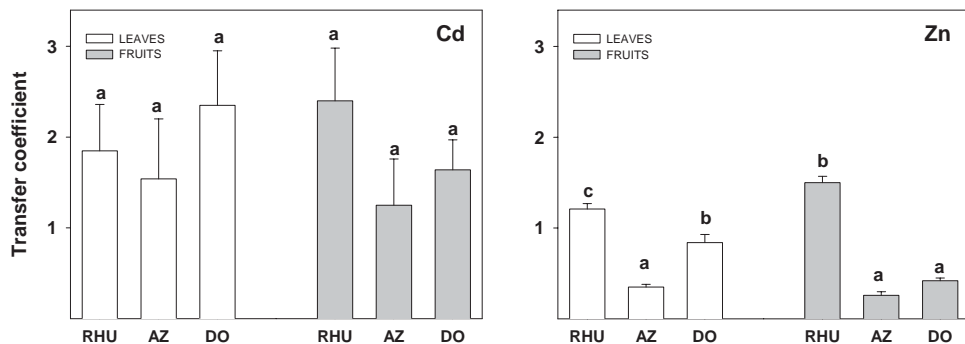
The decreasing concentrations of As and Pb, contrast the high, stable concentrations of Cd and Zn in poplar leaves. This confirms the need for long-term studies to monitor



**Figure 5** Differences in nutrients (Ca, K, Mg, N, P, and S) concentrations in the plant fractions (leaves, catkin stems and fruits) of *Populus alba* L. (spring sampling 2011). For each site, bars (fractions) with the same letter do not differ significantly ( $p < 0.05$ ). Mean values  $\pm$  standard errors are expressed as g/100g of dry matter.

the potential toxicity of residual contamination in the Guadiamar Valley. Moreover, leaf concentrations of Cd and Zn in the last sampling (12 years after the accident), were still much higher than normal values in higher plants ( $0.02$ – $0.5$  mg kg<sup>-1</sup> for Cd and  $27$ – $150$  mg kg<sup>-1</sup> for Zn; Kabata–Pendias and Pendias 1992). In general, long-term datasets are essential resources in ecological and environmental research (e.g., Reinds, Posch, and Vries 2009; Magurran *et al.* 2010).





**Figure 6** Transfer coefficients in poplar leaves (in white) and fruits (in grey) using soil depth 0–20 cm at the three studied sites; sampling in spring 2011. Mean values  $\pm$  standard error. For each fraction (leaves or fruits) bars with the same letter do not differ significantly ( $p < 0.05$ ).

The availability of mineral elements in soil can be best monitored by using the content actually uptaken and accumulated by plants (Madejón *et al.* 2006). Alternatively, phytoavailability can be estimated by chemical extracts that simulate root uptake (Menzies, Donn, and Kopittke 2007). Although, these are indirect methods that are empirically related to the bioavailability of the contaminants to the plants, a single universal soil test to accurately assess risks due to contamination of soils by heavy metals does not exist (McLaughlin *et al.* 2000; Xiao-Lan, Chen, and Shenker 2005).

In the case of trace elements, neutral salt extractants (such as 0.01 M  $\text{CaCl}_2$ ) are widely used and provide the most useful indication (Menzies, Donn, and Kopittke 2007). However this fraction is not easily measured, especially in the case of less mobile elements, such as As and Pb (Madejón *et al.* 2009). To cope with these difficulties, it is common to resort to stronger extractants such as EDTA, although results are not always convincing, as the availability of some trace elements may be overestimated (Vidal *et al.* 1999; McLaughlin *et al.* 2000; Madejón *et al.* 2006). In this study, the leaves of poplar detected a descending trend for As and Pb 12 years after the mine spill. Even though the soil values were below detection limits, we could use poplar leaves as biomonitors to detect a gradual reduction in As and Pb contamination. Therefore, this study corroborates the suitability of poplar leaves to bioindicate soil quality (Madejón *et al.* 2006). This trend was also measured in transfer coefficients from soil to leaves, which decreased with time for As and Pb, but did not vary significantly for the more mobile elements Cd and Zn.

The multivariate analysis of the eight trace elements in poplar leaves during five sampling years indicated a main temporal gradient associated to the contamination of Pb, As and Fe. This trend was similar to that described for spatial variation (five sites) in one single year, 1999 (Madejón *et al.* 2004). The contamination of poplar leaves decreased with time (moving towards the lower pollution region in the PCA space). A secondary trend of variation was site-dependent; concentrations of Zn and Mn in leaf samples increased with time at site DO and remained similar at site AZ.

Most metal localization studies in trees have focused on leaf and stem tissues, with few reporting on metal contents in fruits/seeds. In vascular plants, it is common to have physiological barriers impeding metal translocation to seeds during maturation. In the case of poplar, its ability to accumulate Cd and Zn in its leaves presents a risk to the reproductive structures. The transfer coefficients for Cd and Zn in the non-contaminated zone (RHU)

were relatively high, suggesting that Cd and Zn are able to translocate to the reproductive structures. At the contaminated sites, the transfer coefficients for Cd were significantly greater than those of Zn, indicating a greater mobility and translocation. Based on the toxicity of Cd, this would represent a higher risk to reproductive structures (Kranner and Colville 2011). High concentration of Cd in embryonic or in reserve tissues would have deleterious consequences for seed germination, seedling establishment and colonization success (Kranner and Colville 2011). Despite this relative accumulation of Cd and Zn in green capsules, the poplar seeds may present a physiological barrier at the seed coat, which may protect the embryo from toxic effects (Ernst and Nelissen 2000; Bhatia *et al.* 2003). Further studies to assess the spatial trace element distribution into the seed are advisable; for example, by using micro-Proton-Induced X-ray Emission (Vogel-Mikuš *et al.* 2007).

In an independent study conducted at the same spill site, Domínguez *et al.* (2010b) showed that the altered soil condition (higher level of trace elements and lower pH) originated by the mine-spill enhanced the uptake of several nutrients in afforested *P. alba*. Additionally, a similar 'fertilizing' effect was reported earlier by Murillo *et al.* (1999) on crops that were immediately affected by the mine-spill. In these studies, trace element contamination did not negatively affect mineral nutrition in the studied plants, a positive feature for phytoremediation. Values of macronutrients in leaves, catkin stems and fruits were similar when comparing contaminated and non-contaminated sites, with the exception of higher concentrations of N and Ca in fruits from the non-contaminated site. The nutrients measured in poplar leaves from contaminated sites, were comparable to reported levels for *Populus* species (Mills and Jones 1996): 2–3 for N, 0.37 for S, 0.2–2.6 for K, 0.15–0.4 for P, 1–2.3 for Ca and 0.15–0.7 for Mg (expressed in%).

It is important that the plants chosen for phytostabilization can tolerate high concentrations of trace elements and nutrient imbalances (Bolan *et al.* 2011). In general, white poplar trees used in the phytomanagement of the Guadamar valley showed high survival and growth rates, as well as adequate nutritional status (Domínguez *et al.* 2010a, b). According to the nutrients analysis, white poplar seem to overwhelm this adversity corroborating its suitability for phytostabilization. These results confirm the suitability of *P. alba* to phytoremediate and thus phytostabilize trace element contaminated soil.

The use of accumulator species in phytostabilization could provide an opportunity for trace elements to enter the food web (Domínguez *et al.* 2008; Bolan *et al.* 2011). White poplar could potentially cause an environmental problem with its ability to accumulate Cd and Zn. However, there are several advantages supporting the use of white poplar in the phytostabilization of the Guadamar valley. *P. alba* is the dominant native species in the riparian forests, it is also fast-growing, deep-rooted and tolerant to multiple stress conditions, and it has landscape and cultural values. Fast-growing trees, such as willow, poplar, alder and eucalyptus are commonly used to restore metal contaminated soils (Perttu 1993). Phytostabilization can also benefit carbon sequestration, watershed management, erosion, biomass production, while also improving the landscape for recreational activities (Dickinson *et al.* 2009; Marañón *et al.* 2012).

## CONCLUSIONS

Phytostabilization of contaminated sites requires a monitoring program of trace elements. The results of this study confirmed the use of *Populus alba* as a long term biomonitor

of trace elements in soil. Leaf concentrations of less mobile elements, As and Pb, significantly decreased with time. In contrast, during the 12 years after the mine-spill, concentrations of Cd and Zn accumulated in leaf tissue. Plants suited for phytostabilization should be able to survive, grow and reproduce in contaminated conditions. Fruiting catkins of poplar (measured at the 12th year after the spill) had significant accumulation of Cd and Zn, with possible deleterious consequences for seed germination, seedling establishment and colonization success. In general, the impaired soil conditions at the contaminated sites did not affect the nutritional health of poplar trees. There are advantages of using white poplar to phytostabilize trace metal contaminated soil at the Guadiamar Green Corridor, although more research is needed to understand the effects of trace metals on its reproductive ability and the potential for trace metals to enter food webs.

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Table S1. Trace element concentration in the poplar leaves along the period 1999-2010 (autumn samplings). Mean values  $\pm$  SE (N = 5), expressed as mg kg<sup>-1</sup> dry matter).

Site	Year	As	Cd	Cu	Fe	Mn	Ni	Pb	Zn
AZ	1999	3.05 $\pm$ 0.21	3.53 $\pm$ 0.22	8.39 $\pm$ 0.37	325 $\pm$ 17.4	79.9 $\pm$ 10.2	1.52 $\pm$ 0.09	5.00 $\pm$ 0.47	441 $\pm$ 40
	2000	1.64 $\pm$ 0.14	2.91 $\pm$ 0.37	6.41 $\pm$ 0.37	259 $\pm$ 12.3	64.7 $\pm$ 9.95	0.74 $\pm$ 0.07	2.91 $\pm$ 0.21	409 $\pm$ 64
	2001	1.13 $\pm$ 0.14	3.65 $\pm$ 0.84	9.37 $\pm$ 0.77	293 $\pm$ 35.4	87.8 $\pm$ 15.2	1.34 $\pm$ 0.11	1.60 $\pm$ 0.19	484 $\pm$ 93
	2005	0.51 $\pm$ 0.06	5.15 $\pm$ 0.50	9.15 $\pm$ 0.85	283 $\pm$ 27.4	56.6 $\pm$ 7.84	0.98 $\pm$ 0.15	1.14 $\pm$ 0.05	498 $\pm$ 51
	2010	0.66 $\pm$ 0.16	3.11 $\pm$ 0.28	7.84 $\pm$ 0.26	262 $\pm$ 14.3	56.3 $\pm$ 11.7	1.51 $\pm$ 0.11	0.33 $\pm$ 0.07	283 $\pm$ 39
DO	1999	2.53 $\pm$ 0.21	8.28 $\pm$ 2.20	7.56 $\pm$ 0.44	286 $\pm$ 18.4	72.5 $\pm$ 12.1	1.35 $\pm$ 0.17	5.19 $\pm$ 0.57	832 $\pm$ 174
	2000	2.27 $\pm$ 0.14	5.74 $\pm$ 1.15	5.00 $\pm$ 0.21	242 $\pm$ 9.54	54.9 $\pm$ 9.86	1.00 $\pm$ 0.10	4.34 $\pm$ 0.12	523 $\pm$ 76.0
	2001	1.62 $\pm$ 0.21	7.38 $\pm$ 1.96	7.60 $\pm$ 0.16	228 $\pm$ 15.9	87.1 $\pm$ 16.3	1.30 $\pm$ 0.18	1.92 $\pm$ 0.18	623 $\pm$ 103
	2005	0.73 $\pm$ 0.10	8.80 $\pm$ 2.58	5.84 $\pm$ 0.17	189 $\pm$ 24.7	52.4 $\pm$ 8.32	4.58 $\pm$ 0.19	1.47 $\pm$ 0.13	827 $\pm$ 131
	2010	0.95 $\pm$ 0.06	5.53 $\pm$ 0.51	8.76 $\pm$ 1.19	218 $\pm$ 14.8	94.3 $\pm$ 25.3	2.55 $\pm$ 0.50	1.15 $\pm$ 0.06	860 $\pm$ 111

Table S2. Results of the analysis of variance (ANOVA) with the effects of year, site and their interactions on the concentration of trace elements in *Populus alba* leaves (NS: not significant)

Factor	As		Cd		Pb		Zn	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Year	50.11	0.000	1.04	NS	91.82	0.000	0.94	NS
Site	11.3	0.002	17.54	0.000	12.55	0.001	22.56	0.000
Year x site	4.99	0.002	0.52	NS	1.84	NS	2.32	NS

Table S3. Results of the analysis of variance (ANOVA) with the effects of organ, site and their interactions on the concentration of trace elements in *Populus alba* leaves and fruits (NS: not significant)

Element	Organ		Site		Organ x site	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
As	0.178	NS	0.62	NS	2.03	NS
Cd	6.57	0.005	88.02	0.000	1.56	NS
Pb	1.10	NS	1.85	NS	0.33	NS
Zn	10.23	0.000	83.14	0.000	6.35	0.001
Ca	41.71	0.000	16.39	0.000	15.28	0.000
K	31.99	0.000	9.37	0.001	8.71	0.000
N	225.0	0.000	45.31	0.000	13.37	0.000
Mg	3.86	0.034	1.93	NS	0.90	NS
P	18.94	0.000	25.95	0.000	4.27	0.008
S	24.94	0.000	3.93	0.032	0.81	NS